

Appl. No. 10/718,996  
Amdt. dated Apr. 7, 2009  
Reply to Office Action of Jan. 7, 2009

**Listing of Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Cancelled)
2. (Previously Presented) A method as defined in claim 37, wherein said first capture reagent at said scavenging zone is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies, and complexes thereof.
- 3-4. (Cancelled)
5. (Previously Presented) A method as defined in claim 37, wherein said second capture reagent at said detection zone is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies, and complexes thereof.
6. (Previously Presented) A method as defined in claim 37, wherein said capture reagents at said scavenging zone and said detection zone are substantially identical.
- 7-11. (Cancelled)
12. (Previously Presented) A method as defined in claim 37, wherein said detection probes comprise a substance selected from the group consisting of chromogens, catalysts, luminescent compounds, radioactive compounds, direct visual labels, liposomes, and combinations thereof.
- 13-36. (Cancelled)
37. (Amended) A method for detecting an analyte in a test sample, the method comprising:

i) providing an assay device that comprises:

a sampling pad that defines a scavenging zone in which is non-diffusively immobilized a first capture reagent configured to specifically bind with the analyte;

a conjugate pad that contains detection probes and optional calibration probes, the detection probes being conjugated with a first binding member configured to specifically bind with the analyte; and

a porous membrane in fluid communication with the sampling pad and the conjugate pad, the porous membrane defining a detection zone in which is immobilized a second capture reagent configured to specifically bind with the analyte and a calibration zone within which is immobilized a third capture reagent configured to bind with the detection probes, the calibration probes, or combinations thereof, wherein the detection zone and the calibration zone are located downstream from the sampling pad and the conjugate pad;

ii) contacting the assay device with the test sample, wherein a quantity of the analyte in the test sample less than or equal to a predefined base quantity binds to the first capture reagent at the scavenging zone and a quantity of the analyte in excess of the predefined base quantity binds to the specific binding member of the detection probes to form complexes that flow through the porous membrane and bind to the second capture reagent in the detection zone to generate a detection signal, and wherein the detection probes, the calibration probes, or a combination thereof, flow through the porous membrane and bind to the third capture reagent at the calibration zone to generate a calibration signal;

iii) detecting the intensity of the detection signal and the calibration signal; and

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iv) comparing the intensity of the detection signal to the intensity of the calibration signal, wherein the quantity of the analyte within the test sample in excess of the predefined base quantity is proportional to the intensity of the detection signal calibrated by the intensity of the calibration signal.

38. (Previously Presented) A method as defined in claim 37, wherein the conjugate pad is located downstream from the sampling pad.

39. (Previously Presented) A method as defined in claim 37, wherein the analyte includes an antigen.

40. (Previously Presented) A method as defined in claim 39, wherein the specific binding member of the detection probes includes an antibody.

41. (Currently Amended) A method as defined in claim 40, wherein the first capture reagent and the second capture reagent include antibodies that bind to the same epitope of the analyte ~~an antibody~~.

42. (Previously Presented) A method as defined in claim 39, wherein the antigen includes C-reactive protein.

43. (Previously Presented) A method as defined in claim 37, wherein the test sample is blood or derived from blood.

44. (Previously Presented) A method as defined in claim 43, wherein the analyte includes C-reactive protein.

45. (Previously Presented) A method as defined in claim 44, wherein the pre-defined base quantity is about 10 micrograms of C-reactive protein per milliliter of the test sample.

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46. (Previously Presented) A method as defined in claim 37, wherein the third capture reagent includes a polyelectrolyte having a net charge opposite to the net charge of the detection probes, the calibration probes, or a combination thereof.

47. (Previously Presented) A method as defined in claim 37, wherein the assay device further comprises a wicking pad in fluid communication with the porous membrane and located downstream from the detection zone and the calibration zone.